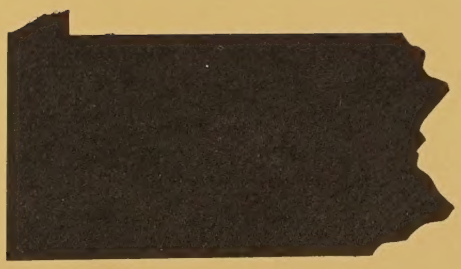
FOREST INSECT AND DISEASE MANAGEMENT / evaluation report

Evaluation of *Bacillus thuringiensis* and a Parasitoid for Suppression of the Gypsy Moth in Pennsylvania 1974



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Summary

The microbial insecticide *Bacillus thuringiensis* Berliner and the parasitoid *Apanteles melanoscelus* Ratz. were evaluated against the gypsy moth, *Porthetria dispar* (L.), infesting an oak forest in Pennsylvania in 1974. The biotic agents were evaluated individually and in combination. Aerial applications of *B. thuringiensis* provided varying degrees of foliage protection and population reduction. Where only the parasitoid was released, foliage protection and population reduction were not significantly different from those in untreated areas. When the parasitoid was released in areas treated with *B. thuringiensis,* additional foliage protection was attained. Only in those areas were larval populations consistently reduced.

B. thuringiensis treatment can provide adequate foliage protection, but additional control measures, such as release of *A. melanoscelus,* will probably be necessary to insure substantial population reduction and eliminate the need for retreatment the following year.

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Introduction

The gypsy moth, *Porthetria dispar* (L.), is a major forest defoliator in the northeastern United States. Efforts are currently being made to develop a pest management system for this insect. Microbial insecticides and parasitoids should be considered as potential candidates for this system. In earlier laboratory studies high concentrations of *Bacillus thuringiensis* Berliner were necessary to cause significantly high mortality of gypsy moth larvae. Field studies have given variable results, but never commercial formulations with more than one application and the use of adjuvants have assisted in making *B. thuringiensis* an effective foliage protectant. However, reduction of the gypsy moth population in the subsequent generation has not been achieved.

An introduced parasitoid *Apanteles melanoscelus* Ratz. has been reported to be effective in low-density gypsy moth populations. Its effectiveness at such densities and its potential reproductive capabilities make this parasitoid a primary choice as a potential component of a pest management system. This parasitoid has also been shown to be unaffected by *B. thuringiensis* sprays.

The objective of this project was to evaluate the field effectiveness of a commercial preparation of *B. thuringiensis* and the larval parasitoid, *A. melanoscelus,* for suppression of the gypsy moth.

Materials and Methods

The project was conducted in a gypsy-moth-infested area in Centre and Union Counties, Pennsylvania. Twelve treatment plots of approximately 10.11 ha (317.9 by 317.9 m) were selected so as to contain as infestation level of 500 to 3500 egg masses per 0.405 ha. The dominant tree species in the experimental area were oaks, 10 to 18 m in height. Ten 0.01-ha circular subplots were established in each treatment block.

A randomized complete-block design was used to evaluate three replications of three treatments and a control. Gypsy moth population density was used as the criterion for the block design.

In the first treatment a commercial preparation of *B. thuringiensis,* Thuricide^{R}-16B,[1] was applied by air at the rate of 8×10^{9} International Units (I.U.) per 0.405 ha. The finished spray material applied to 0.405 ha consisted of 1.89 liters of Thuricide-16B, and 1.89 liters of water. Spraying began when at least 50 percent of the gypsy moth larvae

[1]The use of trade, firm or corporation names is for information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Dept. of Agriculture of any product or service to the exclusion of others which may be suitable.

were in the 2nd instar, and when at least 50 percent leaf expansion had occurred on the white oaks. Two applications were made, the first on May 22 to two plots. A third plot was not sprayed until May 25 because of inclement weather. The second spray was applied June 5 to all plots. Applications were made with a Grumman Ag-Cat^{R}, 450 Hp, equipped with a standard boom and four Beecomist^{R} spinning nozzles (Model 275) with perforated metal sleeve assemblies.

In another treatment the parasitoid *A. melanoscelus* was released. Adult parasitoids of the Yugoslavian strain were obtained from laboratory cultures. Adult parasitoids were maintained in the laboratory at approximately 13^{o}C in darkness and fed with honey and water. Parasitoids were less than three weeks of age at the time of release. Approximately 1000 mated females per plot were released when a majority of the gypsy moth larvae were in the 1st or 2nd instar. These host stages are preferred for oviposition by the parasitoid.

A third treatment evaluated was the combination of *B. thuringiensis* and *A. melanoscelus.* Two aerial applications of Thuricide-16B were made at the same rate and times described. Approximately 1000 mated female parasitoids were also released in these treated plots.

Control plots were also established. Treatments were evaluated on the basis of egg mass numbers before and after treatment, larvae under burlap bands, drop cloth collections, defoliation estimates, parasitoid recovery, spray deposit cards, and a spray residue bioassay.

The number of gypsy moth egg masses was determined by a method similar to that of Connola et al. *(1966).* Prespray egg mass densities ranged from 920 to 3228 egg masses per 0.405 ha (Table 1). Treatment plots were divided into three population densities (low, intermediate and high). Postspray egg masses were counted in November 1974, after leaf drop.

Burlap banding was used to indicate the relative densities of gypsy moth larvae and pupae. Five trees in each subplot were banded, 50 trees per treatment plot. The burlap bands were constructed as described by Yendol et al. *(1973).* All larvae and pupae under or on the burlap and within 7.62 cm below it were counted. Larval density was estimated 15th and 22nd days after spraying. A final count of larvae and pupae was made 36 to 43 days after spraying.

Five cotton drop cloths (0.835 sq m) were set up in a manner described by Connola et al. *(1966).* Drop cloths were cleared of frass and debris immediately after the final spray. The first collection was made 8 to 9 days after spraying. A second collection was made 21 to 22 days after spraying when defoliation was completed. Extraneous material was removed from the drop cloth collections and the frass was air dried and weighed.

Defoliation in each plot was estimated in increments of 20 percent at the end of the larval feeding.

A. melanoscelus cocoons were collected from subplots when the burlap bands were checked. The cocoons were taken to the laboratory where each cocoon was placed in a gelatin capsule, and maintained under a 20 hr photoperiod at 21 to 26^{o}C. Cocoons were checked for parasitoid or hyperparasitoid emergence, and death or diapause when no adult emerged.

Spray cards (white Kromekote^{R}, oil-sensitive) were used to confirm treatment coverage of each plot. They were also placed along borders, separating sprayed and unsprayed areas, to determine possible drift. Cards were positioned under an opening in the forest canopy on 0.3 m wire holders and collected immediately after spraying.

The residual activity or dissipation of Thuricide-16B was also determined. Foliage samples were collected from three sprayed areas and from untreated plots. In each area, two 0.1-ha subplots were sampled. Foliage was collected on the initial spray date and at 1, 3, 5, 8 and 10 days after spraying. Untreated areas were sampled on 0, 3, 8 and 10 days after spraying. Samples were taken at the midcrown, where possible, with specific cardinal points selected at random.

The samples were bioassayed with 2nd instar gypsy moth larvae (4 to 6 mg). Egg masses collected near the experimental site were prepared according to a method described by Yendol et al. *(1973).* Upon eclosion, larvae were transferred to artificial diet *(O'Dell and Rollinson 1966)* and allowed to feed until needed for the bioassay. Leaf material from each subsample area was divided and placed in five 16 oz containers. Ten larvae were placed in each container and maintained at 21 to 26°C with a varying photoperiod. Dead larvae were removed daily.

Unpaired T-tests using unpooled variances were used to analyzed the data from burlap-band counts, drop cloth collections and posttreatment egg mass numbers. This technique was employed because Bartlett's tests indicated heterogenous variances among the plots.

Results and Discussion

Density of larvae and pupae under burlap.

Counts of larvae and pupae on and under burlap bands indicate that *B. thuringiensis* and the combination of *B. thuringiensis* and *A. melanoscelus* reduced the gypsy moth populations of low and intermediate density (initially below 2140 egg masses per 0.405 ha), compared to the control (Table 2). Similar reductions of larval populations have been found where *B. thuringiensis* was applied to infestations with less than 2000 egg masses per 0.405 ha. The application of *B. thuringiensis* significantly reduced each of the 15- and 22-day posttreatment estimates, but not the 36 to 43 day estimate in the high density area (above 2652 egg masses per 0.405 ha).

Population density estimates in the low density area indicated that where only *A. melanoscelus* was released, there was no significant reduction of the gypsy moth populations compared with the control. At the intermediate density, the number of larvae and pupae in the parasitoid release plot was significantly less than the control. When compared with the control, significant increases in the population occurred on the 15- and 22-day posttreatment estimates in the high density plot.

In the high-density population, the numbers of larvae and pupae were significantly reduced by the combination of *B. thuringiensis* and the parasitoid when compared to the other treatments. At the low and intermediate densities, populations that received the combination treatment were lower in the estimate made 36 to 43 days after treatment than those in areas that received only *B. thuringiensis.*

Treatments at each density were analyzed separately because of highly significant ($P<0.01$) interactions shown by the analyses of variance.

Drop Cloth Collections.

Drop cloth collections of larval frass were also used to estimate the relative densities of the population (Table 3). In the initial collections, where *B. thuringiensis* was combined with the parasitoid, significantly lower amounts of frass were obtained than from areas that received the other treatments. When compared with the control and parasitoid-treated plots, the plots treated with *B. thuringiensis* also produced significantly less frass by weight.

In the second observation, the least amounts of accumulated frass again occurred in the combination treatment. These amounts were significantly less than those collected from plots which received other treatments. The *B. thuringiensis* treatment also resulted in significantly less frass by weight than the control and parasitoid treatments.

The addition of the parasitoid to the *B. thuringiensis*-sprayed areas apparently reduced the populations further. This parasitoid may also be useful in mass releases against sparse populations of the gypsy moth *(Weseloh and Anderson 1975).* Our data suggest that the parasitoid could possibly have the same effectiveness on the residual population after treatment with *B. thuringiensis.*

Defoliation estimates.

The control plots were completely defoliated (Table 4). The low and high density populations treated with parasitoids were also completely defoliated, but only 60 to 70 percent defoliation occurred in the intermediate density plots treated with parasitoids. *B. thuringiensis* treatment reduced defoliation to an intermediate level (40 to 50 percent) in two plots and gave considerable foliage protection (0 to 19 percent) in the remaining plot. Considerable foliage protection was also obtained from the combination treatment in each of the three gypsy moth population densities.

Parasitoid recovery.

A total of 97 *A. melanoscelus* cocoons were collected from all of the plots 36 to 43 days after spraying. They were used to determine survival and percentage of diapause. The occurrence of parasitoid pupae in every plot including the control, suggests that a natural population could have been present or that parasitoids eventually dispersed into the untreated and *B. thuringiensis*-treated plots from the release areas. There were no significant differences among treatments in emergence, diapause, or mortality. Of the parasitoid samples, 19.3 percent emerged, 23.7 percent entered diapause, and the remaining 57 percent died.

Spray deposit cards.

Spray deposit cards positioned between sprayed and unsprayed areas indicated that some spray drifted onto the area of intermediate population density treated with parasitoids. This error in spray application may offer an explanation of why a portion of this plot was not completely defoliated.

Spray deposits were recovered from all sprayed areas. The deposit showed a mean of 21 droplets per cm^2 and a droplet size averaging 156 μm.

Final egg mass determination.

The number of egg masses found after treatment was substantially higher in the control plots and plots with parasitoid treatment than the pretreatment estimates (Table 5). Plots treated with *B. thuringiensis* had significantly fewer egg masses than the control at low and intermediate densities, but there was little or no reduction from pretreatment levels. The high-density plot treated with *B. thuringiensis* showed no significant reduction from the control or the pretreatment estimate.

The combination treatment resulted in significantly fewer egg masses than the control plots had at all densities. Pretreatment egg mass numbers were substantially reduced in intermediate and high-density plots.

Spray residue bioassay.

The residual activity of *B. thuringiensis* is summarized in Figure 1. At least 92 percent of larvae which were fed foliage collected on the same day as the spray application died. Before the second application, the *B. thuringiensis* remaining on the foliage killed only 14.6 percent. The residual activity of *B. thuringiensis* dissipated more rapidly after the first application than after the subsequent application.

Foliage from the first day of the second application produced 92 percent mortality. On the 10th day after application, its activity was still at almost 50 percent of its original level. The mortality in the control plot ranged from 1 to 10 percent, and could not be attributed to a viral or bacterial infection.

Numerous factors control the persistence of *B. thuringiensis* in the field. Investigations by Cantwell and Franklin *(1966)* showed that moist, unprotected spores of *B. thuringiensis* were rapidly killed by exposure to sunlight. In some cases the half-life of the bacterial spore may be closely related to the pathogenicity half-life of the formulation *(Pinnock et al. 1971),* although this relationship may depend on the specific target insect *(Sutter et al. 1966; Summerville et al. 1970).* Rainfall undoubtedly contributes greatly to the persistence of the microbial material in the field.

Conclusions

Release of the parasitoid *A. melanoscelus* in areas treated with *B. thuringiensis* caused a further reduction in residual populations. This treatment produced lower larval populations, as indicated by drop cloth collections of larval frass, and less defoliation.

Larval and pupal densities estimated from burlap counts and final egg mass determinations indicated that the high density combination treatment plot had a significantly lower population than the other high density treatments. Low and intermediate density combination treatments had significant reductions when compared to the parasitoid or control plots of the same density.

Where only *B. thuringiensis* was applied, results were variable. The addition of the parasitoid consistently reduced populations further and substantially increased foliage protection when compared to the control or parasitoid treatment.

B. thuringiensis treatment of gypsy moth populations below 3000 egg masses per 0.405 ha will provide some degree of foliage protection and population reduction. The results of this project suggest that additional control measures would probably be necessary to reduce the population substantially and eliminate the need for retreatment the following year. Other parasitoids, pathogens, or chemicals, which may be more effective in reducing the residual population after *B. thuringiensis* treatment should be tested.

Acknowledgment

Appreciation is given to Dr. Richard Craig of the Horticulture Department, The Pennsylvania State University, who gave consultation on the statistical analyses of the data.

Pesticide Precautionary Statement

This publication reports evaluations involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife – if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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Table 1. — *Pretreatment estimates of gypsy moth egg masses categorized into three population densities*

Treatment	Egg Masses/0.405 hectare[a]		
	Low Density	Intermediate Density	High Density
Control	1548	2140	2652
A. melanoscelus	1464	1940	2988
B. thuringiensis	1064	1276	2996
B. thuringiensis + *A. melanoscelus*	920	2112	3228

[a]Egg mass density estimates were determined from ten 0.01-hectare subplots in each 10.11-hectare plot.

Table 2. — *Effect of various treatments on gypsy moth larval and pupal populations as determined by the burlap band method*

Treatment	Mean number of larvae and pupae/tree[a]								
	Low density			Intermediate density			High density		
	Days post-treatment			Days post-treatment			Days post-treatment		
	15	22	36-43	15	22	36-43	15	22	36-43
Control	13.1a	57.6a	97.5a	14.2a	30.1a	78.9a	20.1a	50.1a	84.2a
A. melanoscelus	14.8a	37.3a	82.5a	11.3b	12.1b	60.5b	38.7b	121.7b	90.5a
B. thuringiensis	.9b	2.7b	50.2b	.8c	.7c	20.2c	1.6c	3.7c	91.3a
B. thuringiensis + *A. melanoscelus*	1.4b	1.6b	37.2b	.5c	.9c	15.3c	.2d	3.7c	29.2b

[a]Means in the same column not followed by the same letter are significantly different at the 5% level. (Unpaired T-Test using unpooled variances.)

Table 3. — *The amount of frass collected in drop cloths situated in various treatments*

Treatment	Mean weight (g) of frass[a,b,c]	
	Days posttreatment	
	8 – 9	21 – 22
A. melanoscelus	6.157 a	25.930 a
B. thuringiensis	4.547 a	20.671 a
B. thuringiensis + *A. melanoscelus*	0.463 b	4.776 b
	0.095 c	0.821 c

[a]Means based on 15 drop cloths per treatment (5 drop cloths per plot).

[b]Observations were pooled from the three densities because there were no significant replication or interaction effects.

[c]Mean in the same column not followed by the same letter are significantly different at the 5% level (Unpaired T-test using unpooled variances).

Table 4. — *The degree of defoliation occurring in plots treated with B. thuringiensis and A. melanoscelus*

Treatment	Estimated % Defoliation		
	Low Density	Intermediate Density	High Density
Control	80–100	80–100	80–100
A. melansocelus	80–100	60– 79	80–100
B. thuringiensis	40– 59	0– 19	40– 59
B. thuringiensis + *A. melanoscelus*	9– 19	0– 19	0– 19

Table 5. — *Posttreatment estimates of gypsy moth egg masses and the difference between pre- and post-treatment estimates*

Treatment	Egg masses/0.405 hectare[a] and % change					
	Low density		Intermediate density		High density	
	Masses[b]	%	Masses	%	Masses	%
Control	6796a	+339.0	4632a	+116.4	4880a	+84.0
A. melanoscelus	4604a	+214.5	5620a	+189.7	3704a	+24.0
B. thuringiensis	1404b	+ 32.0	1236b	– 3.0	4320a	+44.2
B. thuringiensis + *A. melanoscelus*	2224b	+141.7	784b	– 62.9	564b	–82.5

[a]Egg mass density estimates were determined from counts made in ten 0.01-hectare subplots in each 10.11-hectare plot.

[b]Means in the same column not followed by the same letter are significantly different at the 5% level (Unpaired T-Test using unpooled variances).

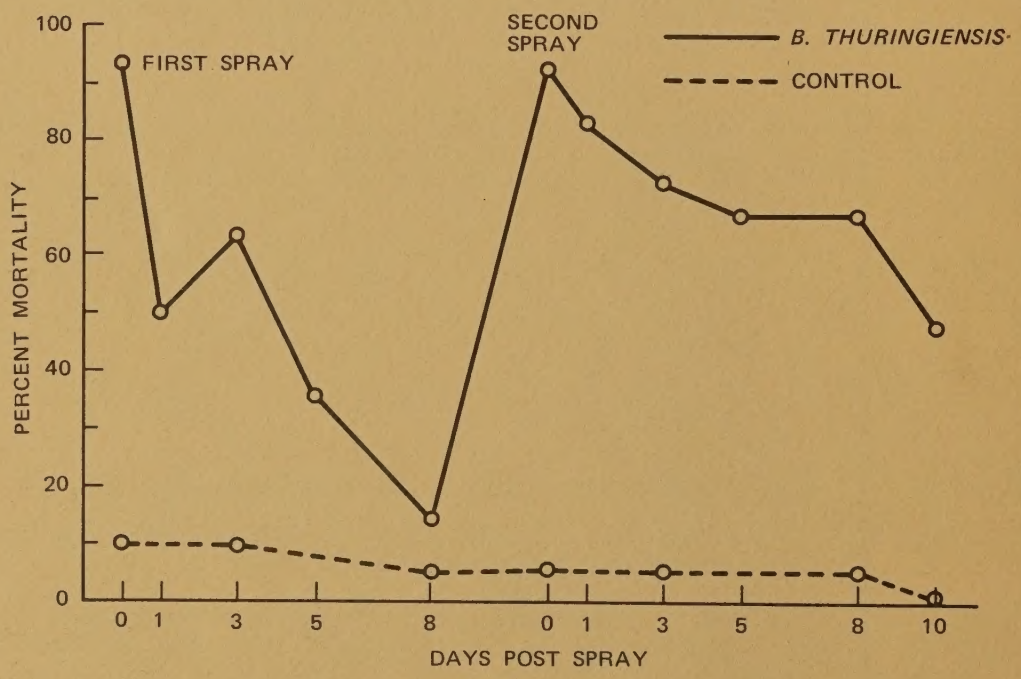

FIGURE 1. DISSIPATION OF INSECTICIDAL ACTIVITY OF *B. THURINGIENSIS* APPLIED BY AIR TO THE EXPERIMENTAL AREA.